Kidney International, Vol. 68 (2005), pp. 2019–2028

HORMONES – CYTOKINES – SIGNALING

Growth hormone promotes glomerular lipid accumulation in bGH mice

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Growth hormone promotes glomerular lipid accumulation in bGH mice.

Background. Bovine growth hormone (bGH) transgenic mice develop progressive glomerulosclerosis and exhibit abnormalities in hepatic lipid metabolism. We have previously shown that growth hormone up-regulates the low-density lipoprotein (LDL) receptor and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) in mouse mesangial cells. However, a role of lipid abnormalities in bGH kidney disease has not yet been demonstrated.

Methods. Groups of bGH mice (5 and 11 months old) presenting with, respectively, moderate and severe degrees of glomerulosclerosis were compared to age-matched controls. Neutral lipid content in kidney cortex was determined by oil red-O staining, serum cholesterol, and triglycerides by enzymatic assays, relative mRNA expression of LDL receptors, HMGR, and scavenger receptor by real-time reverse transcription-polymerase chain reaction (RT-PCR), and HMGR protein expression by immunoblotting. Two younger (5 and 12 weeks old) groups of mice were used to study scavenger receptor expression at earlier time points.

Results. Serum cholesterol was significantly increased in bGH mice at 5 months, but triglycerides were lower than control levels at both 5 and 11 months. Renal cortex HMGR expression was elevated at the mRNA but not at the protein level in the 11-month-old bGH group compared to controls. However, glomerular neutral lipid staining and scavenger receptor mRNA expression were markedly increased in all bGH mice, including those at 5 weeks of age compared to respective controls.

Conclusion. The bGH mouse exhibits an increased mesangial lipid content and elevated scavenger receptor mRNA expression as early as at 5 weeks of age, suggesting that an increased kidney uptake of oxidized LDL could play a role in the development of glomerulosclerosis in this mouse model.

Key words: glomerulosclerosis, cholesterol, triglycerides, HMG-CoA reductase, low-density lipoprotein receptor, scavenger receptor, real time RT-PCR.

Received for publication August 11, 2004
and in revised form March 21, 2005, and May 3, 2005
Accepted for publication June 1, 2005

Glomerulosclerosis is characterized by glomerular lesions, which include partial or total replacement of the glomerular tuft by a fibrous scar, resulting in progressive loss of renal function [1]. It constitutes a common feature of most chronic kidney diseases, including diabetic nephropathy. Of the several animal models of glomerulosclerosis in widespread use, the bovine growth hormone (bGH) transgenic mouse is of particular interest as it develops glomerular lesions that bear a marked resemblance to those present in human diabetic nephropathy [2]. Because of the progressive nature of the disease, the bGH mouse has become a model to study the pathophysiology of glomerulosclerosis. Furthermore, the clinical observation that in acromegaly, abnormally high circulating levels of growth hormone are accompanied by an increase in albuminuria and urinary excretion of glycosaminoglycans, early markers of glomerular injury, strongly supports the involvement of elevated growth hormone in the pathogenesis of glomerular disease [3]. The role of growth hormone in the development of glomerulosclerosis has also been demonstrated in dwarf models of diabetes and subtotal nephrectomy, where growth hormone deficiency was shown to markedly attenuate the severity of glomerular injury [4–7].

It has been reported recently that excess growth hormone in the bGH transgenic mouse leads to hypercholesterolemia and several abnormalities in hepatic lipid metabolism [8, 9], but the involvement of lipids in the development of glomerulosclerosis has not yet been studied in this model. Histopathologic similarities between

glomerulosclerosis and atherosclerosis suggest that subsequent to the initial hormonal, metabolic or hemodynamic insult, the development of glomerulosclerosis follows a course analogous to that of lipid-induced vascular disease [10–13]. As in atherosclerosis, an increased presence of macrophages in the glomerular area, followed by increased glomerular lipid deposition, mesangial cell proliferation and extracellular matrix expansion have been demonstrated in several types of experimental and clinical nephropathies [10, 11, 14]. Furthermore, the demonstration that glomerular low-density lipoprotein (LDL) deposits induce both mesangial cell proliferation [15–18] and extracellular matrix accumulation [19] supports the idea that lipid deposition may underlie the development of glomerulosclerosis.

In the presence of hyperlipidemia, an increased scavenger receptor–mediated uptake of oxidized LDL by glomerular macrophages leads to the development of glomerular foam cells [12, 20]. In addition, cultured human mesangial cells have been shown to accumulate lipids in the presence of high concentrations of LDL through both class A scavenger receptor [21, 22] and LDL receptor [23–25].

Despite the recognized association between hyperlipidemia and both atherosclerosis and glomerulosclerosis, there is evidence that mesangial lipid accumulation in glomerular diseases may occur independently of serum lipid levels, related instead to the renal up-regulation of LDL receptor and scavenger receptor [26, 27].

We have previously shown that growth hormone increased LDL receptor and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) transcript levels in mouse mesangial cells cultured in the absence of lipoprotein [28]. These results suggest a model in which growth hormone mediates the development of glomerulosclerosis in part by a direct mesangial up-regulation of genes involved in lipid metabolism. In the present study, we extended our in vitro studies to an in vivo model of glomerulosclerosis (bGH transgenic mouse) to examine the influence of growth hormone on the renal expression of genes involved in the regulation of lipid synthesis and uptake during the course of glomerulosclerosis. We first measured the renal expression of LDL receptor, HMGR, and scavenger receptor at both moderate and severe stages of chronic kidney disease, respectively, 5- and 11-month-old animals. In addition, the levels of serum creatinine, cholesterol, and triglycerides, as well as accumulation of intraglomerular lipid were compared between bGH and age-matched control animals. In a second study, four additional groups of animals (bGH and nontransgenic at 5 and 12 weeks of age, five animals/group) were included specifically to ascertain the earliest occurrence of a difference in glomerular lipid accumulation and scavenger receptor expression between the bGH and nontransgenic groups.

METHODS

Animals

bGH transgenic mice and nontransgenic littermates were provided by Dr. John J. Kopchick (Edison Biotechnology Institute, Ohio University, Athens, OH, USA). The animals were housed one per cage, with free access to water and mouse chow (rat/mouse standard diet) (Harlan Teklad, Madison, WI, USA) in alternating 12-hour periods of light and dark. A total of 28 male mice were divided into age group I (5 months old) (nine nontransgenic and 10 bGH) and age group II (11 months old) (five nontransgenic and four bGH), representing moderate and severe stages of glomerulosclerosis, respectively [29]. In a second study, four groups of animals were euthanized at a younger age (bGH and nontransgenic mice, 5 and 12 weeks of age, five animals/group). All animal procedures were carried out in accordance with the regulations of the USUHS Laboratory Review Board and with the Guide for the Care and Use of Laboratory Animal, Institute of Laboratory Animal Resources, National Research Council, 1996.

Animals were weighed and euthanized by decapitation following sedation with intraperitoneal injection (12 μL/g body weight) of Avertin (25 g tribromoethanol/12.5 mL tertiary amyl-alcohol) at 1/40 dilution in saline [30]. All mice were euthanized in the morning to avoid the effects of diurnal variation. Total blood was collected and allowed to clot, followed by separation of serum, which was kept at −80°C until analysis. Kidneys were quickly excised and weighed. Samples of kidney cortex were collected for RNA extraction and for preparation of histology sections (frozen and paraffin-embedded). In the second study, prior to euthanasia the 5-week-old nontransgenic and bGH animals were grouped in separate metabolic cages (five animals per group) for a period of 3 hours in 3 consecutive days for urine collection and further assessment of proteinuria.

Light microscopy

A coronal section of the right kidney was fixed in 10% buffered formalin and embedded in glycol methylacrylate. Sections (3 μm) were stained with periodic acid-Schiff (PAS) and examined by an independent investigator blinded to the study to determine the degree of glomerular injury, indicated by a glomerulosclerosis score. The scoring system was based on the modified Banff classification [31] using data collected from the analysis of 20 glomeruli/mouse that were randomly scanned proceeding from the outer cortex to the juxtaglomerular zone in a serpentine fashion. First, each glomerulus was graded (0 to 4) according to the percentage of sclerosis/glomerular area, grade 0 being given to glomeruli with no sclerosis and grade 4 to obsolescent glomeruli. Subsequently, a final score was attributed

to each animal according to the percentage of sclerotic glomeruli previously graded, score 0 being given to animals with less than 10% grade 1 glomeruli and score 4 to animals with >75% grade 3 glomeruli and ≥10% grade 4 glomeruli.

Neutral lipid staining

Fresh kidney tissue was immediately frozen in 22-oxacalcitriol (OCT) compound (Miles Scientific, Naperville, IL, USA), and cryostat sections of 14 μm were fixed in 40% formalin for 5 minutes. A fresh working solution of oil red-O was prepared by dilution of the oil red-O stock solution (5 g/L in 98% isopropanol) in distilled H_2O in a ratio of 3:2. The working solution was allowed to stand for 10 minutes, after mixing and was filtered in a 0.45 μ filter. Subsequently, sections were stained in oil red-O for 10 minutes, washed in tap water and counterstained with Harris hematoxylin solution followed by dipping in ammonia water (28% ammonium hydroxide). The slides were allowed to dry and were mounted with Vectashield mounting medium (Vector Laboratories, Burlingame, CA, USA).

Light microscopy of oil red-O–stained sections of kidney cortex was performed and the number of glomeruli with positive neutral lipid staining was determined to calculate the percentage of oil red-O–positive glomeruli per total number of glomeruli for each section. The average percentage of neutral lipid-positive glomeruli was compared between bGH and respective age-matched control groups.

Biochemical analyses

Cholesterol and triglyceride serum concentrations were determined using the Infinity® Kit from Sigma Diagnostic (St. Louis, MO, USA), following the manufacturer's protocol. Serum creatinine was measured by a colorimetric assay based on the Jaffé reaction using a commercial kit (Sigma Diagnostics). Proteinuria was measured in urine samples collected from the 5 week-old bGH and nontransgenic mice by reagent strips (Multistix 10SG) (Bayer Co., Elkhart, IN, USA).

RNA extraction and real time reverse transcription-polymerase chain reaction (RT-PCR)

Samples of renal cortex were homogenized and total RNA was extracted using the Rneasy Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. This procedure included a DNAse step to eliminate genomic DNA contamination. Total RNA concentration was determined by absorbance at 260 nm. Aliquots of 100 ng of each sample in duplicate were employed to determine a relative quantification of target transcript using real-time RT-PCR with the TaqMan® One-Step RT-PCR Master Mix Reagents (Applied Biosystems, Foster City, CA, USA) and the ABI 7700 Sequence Detector System (Applied Biosystems). The final concentrations of primers and probes in the reaction were optimized for multiplex real time RT-PCR as follows: HMGR forward and reverse primers 150 nmol/L, probe 150 nmol/L; LDL receptor forward and reverse primers 300 nmol/L, probe 200 nmol/L; scavenger receptor forward and reverse primers 300 nmol/L, probe 250 nmol/L; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward and reverse primers 40 nmol/L, probe 200 nmol/L. All reactions were carried out at 48°C for 30 minutes (RT step), followed by one cycle of 95°C for 10 minutes and 40 cycles of 95°C for 15 seconds and 60°C for 1 minute.

Each target transcript was coamplified with GAPDH in a multiplex RT-PCR assay. Normalization was calculated by the difference between target and GAPDH C_T levels (ΔC_T). Subsequently, a quantification of target mRNA in bGH mice relative to control animals was calculated by the difference between ΔC_T values ($\Delta\Delta C_T$) and expressed as fold change using the formula $2^{-\Delta\Delta CT}$ (User Bulletin # 2) (PE Applied Biosystems).

Primers and TaqMan® probes

Primers and TaqMan® probes specific for mouse HMGR, LDL receptor, scavenger receptor (recognizing class A types I and II) were designed using Primer Express software (Applied Biosystems). Specificity of transcript amplification was verified using a nucleotide Basic Local Alignment Search Tool (BLAST) (National Center for Biotechnology Information, NIH, Bethesda, MD, USA). Probes for target genes were labeled with a reporter dye (FAM) at the 5′ end and a quencher (TAM) at the 3′ end, and were synthesized at the Core Laboratory of the Center for Biologics Evaluation and Research (CBER) (Food and Drug Administration, Bethesda, MD, USA). A set of primers and TaqMan® probe for the housekeeping gene GAPDH was obtained from PE Applied Biosystems. The GAPDH probe was labeled with the fluorophore VIC at the 5′ end and TAM at the 3′ end. The sequences of primers and probes for HMGR, LDL receptor and scavenger receptor are shown in Table 1.

Western blot

Total protein was extracted from kidney cortex samples using glass homogenizers with ice-cold buffer containing 50 mmol/L Tris-HCl, pH 8.0, 150 mmol/L NaCl, 0.5% sodium deoxycholate, 1% NP-40, 0.1% sodium dodecyl sulfate (SDS), 5 mmol/L ethylenediaminetetraacetic acid (EDTA), to which was added dithiothreitol (DTT) to a final concentration of 1 mmol/L and one pill of protease inhibitors (Roche Pharmaceuticals, Nutley, NJ, USA) per 10 mL of buffer. Tissue homogenate was centrifuged at

Table 1. DNA sequence of primers and probes used in real-time reverse transcription-polymerase chain reaction (RT-PCR)

	Forward primer	Reverse primer	Probe
HMGR	tctggcagtcagtgggaactatt	cctcgtccttcgatccaatttt	caccgacaagaagcctgctgcca
LDL receptor	gctccataggctatctgctcttca	ctgcggtccagggtcatc	caaccgccacgaggtccgga
Scavenger receptor	catgaacgagaggatgctgact	aggaagggatgctgtcattga	cagttcagaatccgtgaaatttgacgcac

HMGR is 3-hydroxy-3-methylglutaryl coenzyme A reductase; LDL is low-density lipoprotein. Sequences are oriented in the 5′-3′ direction. Probes were designed according to the TaqMan™ technology, labeled with the fluorophores FAM and TAM, respectively at the 5′ and 3′ ends.

Table 2. Kidney weight, glomerulosclerosis (GS) scores and serum lipids of bovine growth hormone (bGH) compared to control mice at 5 and 11 months of age

					Serum concentration	
	Body weight *g*	Kidney weight *g*	Kidney weight/body weight *mg/g*	GS score mean (min-max)	Cholesterol	Triglycerides
5 months						
bGH	50.8 ± 1.1[a]	0.64 ± 0.09[a]	12.7 ± 2.0	3.3 (2.0-4.0)[a]	181.2 ± 9.0[a]	85.6 ± 8.0
Nontransgenic	33.5 ± 1.1	0.31 ± 0.06	9.1 ± 1.8	0.3 (0.0-1.0)	135.8 ± 6.6	120.2 ± 13.8
11 months						
bGH	51.8 ± 1.1[a]	0.56 ± 0.05[a]	10.9 ± 1.0[a]	4.0 (4.0-4.0)[a]	114.3 ± 8.6	50.6 ± 7.7[b]
Nontransgenic	35.6 ± 1.5	0.25 ± 0.02	7.4 ± 0.7	0.9 (0.0-1.0)	136.6 ± 5.1	122.4 ± 17.5

Results in mean ± SEM. Level of significance $P < 0.05$.
[a]Value significantly higher compared to age-matched nontransgenic animals.
[b]Value significantly lower compared to age-matched nontransgenic animals.

10,000 × *g* at 4°C for 20 minutes. The supernatant was collected and protein concentration was determined using the Bio-Rad Protein Assay Dye Reagent (Bio-Rad Laboratories, Hercules, CA, USA).

Aliquots of 30 µg of protein were added to Bio-Rad SDS sample buffer (Bio-Rad Laboratories), 1× NuPAGE Reducing Agent (Invitrogen Corp., Carlsbad, CA, USA) in a total volume of 20 µL and heated at 70°C for 10 minutes. Samples were loaded on 4% to 12% NuPAGE Novex Bis-Tris precast gels (Invitrogen Corp.) with NuPAGE 3-[N-morpholino] protein sulfonic acid (MOPS) SDS Running Buffer (Invitrogen Corp.) containing 0.25% NuPAGE Antioxidant (Invitrogen Corp.) and were subjected to electrophoresis at 125 V. Subsequently, proteins were transferred from the gel to a polyvinylidine difluoride (PVDF) membrane with NuPAGE Transfer Buffer (Invitrogen Corp.) containing 20% methanol and 0.1% NuPAGE Antioxidant (Invitrogen Corp.).

The blotted membranes were incubated in blocking solution [5% fat-free milk and 0.1% Tween-20 in phosphate buffered saline (PBS)] for 1 hour at room temperature, followed by incubation with rabbit antimouse HMGR antibody (Upstate USA, Charlottesville, VA, USA) diluted 1:500 in blocking solution for 2 hours at 4°C. Subsequently, the blotted membrane was washed three times in PBS with 0.1% Tween-20 for 10 minutes at room temperature, followed by incubation with goat antirabbit horseradish peroxidase (HRP)–conjugated antibody (Pierce Chemical Co., Rockford, IL, USA) diluted 1:5000 in blocking solution for 90 minutes at room temperature. After three washes of 10 minutes each, the membrane was immersed in the SuperSignal West Pico chemiluminescent substrate (Pierce Chemical Co.) with constant agitation for 5 minutes and exposed to a high-performance chemiluminescence film (Hyperfilm ECL) (Amersham Biosciences, Piscataway, NJ, USA) for 5 to 10 seconds. The film was developed and the image was scanned to a computer for densitometric analysis using NIH Image software (National Institutes of Health, Bethesda, MD, USA).

Statistical analyses

Glomerulosclerosis scores were analyzed by a nonparametric test (Kruskal-Wallis) followed by Dunn's post test. Other parameters were compared by one-way analysis of variance (ANOVA) followed by Bonferroni test using InStat software (Graph-Pad, San Diego, CA, USA). For real-time RT-PCR assays, significance was determined by a comparison of the ΔC_T values calculated for each experimental group rather than from the formula-derived ($2^{-\Delta\Delta CT}$) fold change from control. The level of significance accepted was $P < 0.05$.

RESULTS

The influence of excess growth hormone on kidney morphology in the bGH transgenic mouse model at 5 and 11 months is illustrated in Table 2 and Figure 1A. All bGH transgenic mice exhibited a significant increase in body and kidney weight compared to age-matched nontransgenic controls. The kidney to body weight ratio was approximately 1.4-fold higher in bGH compared to nontransgenic mice in both age groups, but a statistically significant difference was achieved only in the 11-month-old

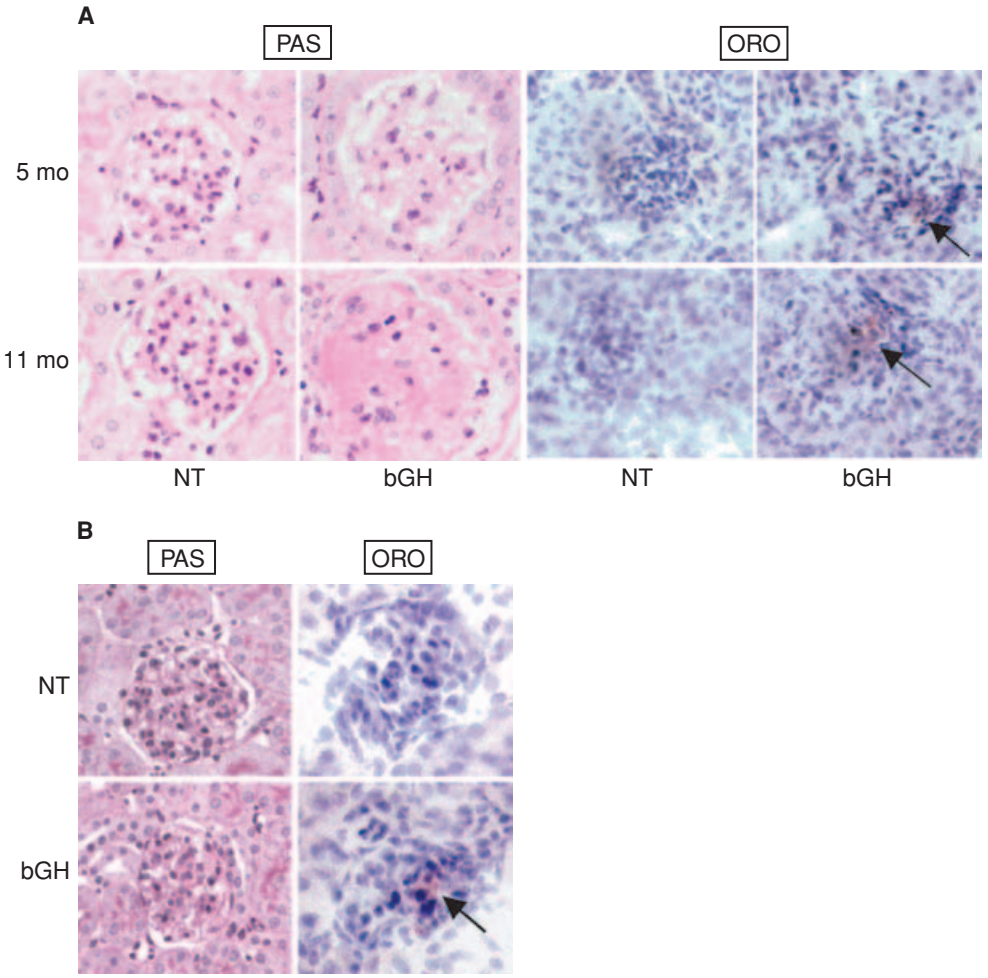

Fig. 1. Photomicrograph of representative glomeruli from bovine growth hormone (bGH) and nontransgenic (NT) mice at 5 and 11 months of age (*A*) and at 5 weeks of age (*B*), stained with periodic acid-Schiff (PAS) reagent and with oil red-O (ORO). (A) Note the PAS-stained extracellular matrix accumulated within bGH glomeruli with collapsed capillary loops at 5 months and particularly at 11 months of age. In contrast, glomeruli of age-matched nontransgenic animals exhibit open capillary loops and no extracellular matrix accumulation in the mesangium. Neutral lipid accumulation indicated by oil red-O–positive staining (arrows) was present in a significantly higher percentage of glomeruli in bGH compared to nontransgenic mice at both 5 and 11 months. (B) At 5 weeks of age, there was no evidence of PAS-stained extracellular matrix accumulation at light microscopy examination in bGH compared to control mice. However, the percentage of glomeruli showing positive staining for neutral lipid (arrow) was significantly increased in bGH compared to age-matched controls (magnification 40×).

group. Glomeruli from both groups were scored as described in the **Methods** section, and bGH mice at both 5 and 11 months old were found to have significantly elevated glomerulosclerosis scores compared to their age-matched controls (Table 2). Serum creatinine levels were not significantly different among groups (0.51 ± 0.07 and 0.40 ± 0.03, respectively, for nontransgenic and bGH at 5 months; 0.36 ± 0.03 and 0.35 ± 0.05, respectively, for nontransgenic and bGH at 11 months) (values are mean ± SEM).

The influence of the bGH genotype on the serum lipid profile is also illustrated in Table 2. Serum cholesterol levels were significantly higher (by approximately 25%) in bGH mice compared to their control counterparts at 5 months of age, but no difference in cholesterol levels between bGH and nontransgenic mice was detected at 11 months. Furthermore, serum triglycerides exhibited a trend to decrease in the bGH group at 5 months and by 11 months were significantly reduced in bGH compared to age-matched nontransgenic mice.

Despite the finding of normal cholesterol and low triglyceride serum levels in bGH mice at 11 months, positive oil red-O staining, a reflection of neutral lipid content, was nevertheless markedly increased in glomeruli of bGH mice compared to controls in both the 5- and 11-month-old age groups. A total of 20 ± 10 glomeruli/section were analyzed, and the percentage of glomeruli with positive oil red-O staining was significantly increased in the bGH group ($P < 0.05$, ANOVA followed by Tukey-Kramer test for multiple comparisons) (values are mean ± SD) at both 5 months (46.8 ± 30.1 vs. 0.8 ± 2.4, respectively, for bGH and

nontransgenic) and 11 months (73.5 ± 18.8 vs. 7.7 ± 11.6, respectively, for bGH and nontransgenic). Representative PAS- and oil red-O–stained sections of renal cortex illustrating the advanced degree of extracellular matrix accumulation and glomerular neutral lipid deposition in bGH compared to nontransgenic mice at both 5 and 11 months of age are shown in Figure 1A.

Most of the oil red-O staining was associated with glomeruli rather than with tubular or interstitial areas. The disparity between oil red-O staining and serum levels of cholesterol at 11 months suggested that the bGH-related increase in glomerular lipid may be more directly related to the influence of growth hormone on lipid-handling pathways than it is to circulating lipid levels. To examine whether the glomerular neutral lipid deposition in bGH mice occurred as a consequence of or as a precursor of the sclerotic process, we studied an additional group of animals at a very young age (5 weeks), in which there was no discernible difference between bGH and nontransgenic in PAS-stained mesangial matrix. Despite the absence of any noticeable sign of glomerulosclerosis, the percentage of glomeruli with positive staining for oil red-O was significantly increased in bGH compared to age-matched controls (76.2 ± 11.5 vs. 1.0 ± 2.2, respectively, for bGH and nontransgenic) (values are mean ± SD) at 5 weeks of age. Representative PAS- and oil red-O–stained sections for nontransgenic and bGH mice at 5 weeks are illustrated in Figure 1B. The absence of evident glomerular scarring at 5 weeks was supported by our findings that proteinuria was absent in bGH as was in nontransgenic mice.

To further assess the role of growth hormone in directly affecting glomerular lipid, we employed real-time PCR to measure the growth hormone effect on enzymes and receptors regulating mesangial cholesterol synthesis and uptake. Our results showed that HMGR expression in the 5-month-old bGH mice was not significantly different from age-matched controls, but by 11 months transcript levels of HMGR were increased by approximately sixfold in bGH compared to their control counterparts (Fig. 2A). However, there was no significant difference in immunoreactive HMGR protein between bGH and control animals at either 5 or 11 months as measured by densitometric analysis of Western blots (Fig. 2B). Furthermore, the mRNA expression of LDL receptor did not show a significant difference between bGH and nontransgenic at either time point (Fig. 3A), suggesting that the activity of the HMGR and LDL receptor in the kidney cortex was not significantly affected by the increased serum cholesterol in the 5-month-old bGH mice. In contrast, scavenger receptor transcript levels were markedly elevated by eightfold in bGH mice at 5 months of age and this growth hormone–related increase was sustained (fourfold above control) through the course of the kidney disease up to 11 months of age compared with age-matched controls (Fig. 3B).

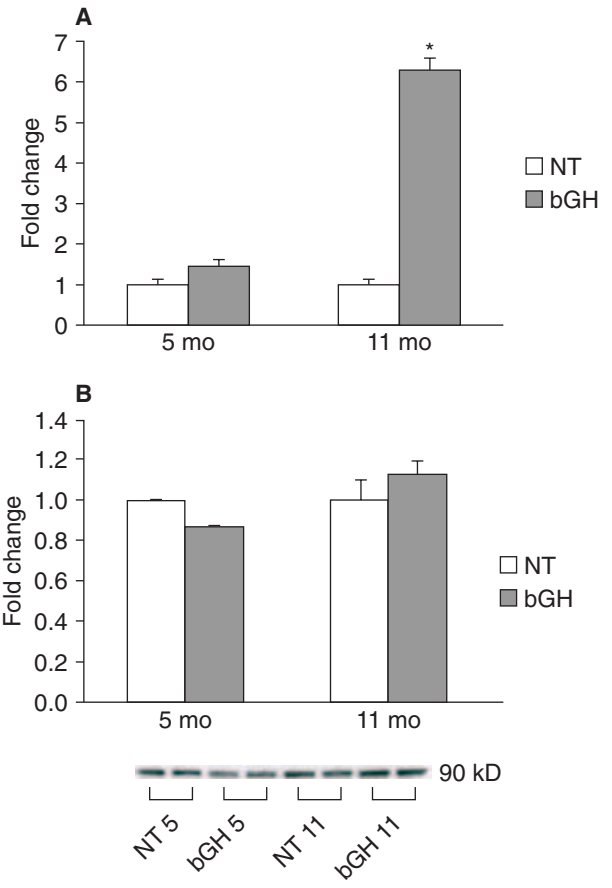

Fig. 2. 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (HMGR) mRNA and protein expression in bovine growth hormone (bGH) and nontransgenic (NT) mice at 5 and 11 months. (*A*) Transcript levels measured by real-time reverse transcription-polymerase chain reaction (RT-PCR) are significantly increased in bGH mice at 11 months, but not at 5 months compared to respective age-matched controls. Bars represent fold change of bGH values over age-matched nontransgenic (calculated by the formula $2^{-\Delta\Delta CT}$) and are mean ± SEM of three independent experiments. Significant differences between groups were determined by analysis of variance (ANOVA) followed by Bonferroni multiple comparisons on ΔC_T values. (*B*) Representative Western blot showing immunoreactive HMGR (90 kD band) in protein samples extracted from kidney cortex of bGH and nontransgenic mice at 5 and 11 months. A quantitative analysis (graph) shows no significant difference in immunoreactive HMGR content between bGH and age-matched nontransgenic mice. Values are mean ± SEM of two independent experiments. $^*P < 0.05$ vs. nontransgenic at 11 months.

Subsequent to the initial study of scavenger receptor expression in 5- and 11-month-old (i.e., 20 and 44 weeks) mice, we extended these experiments to include bGH mice and age-matched controls at 5 and 12 weeks (Fig. 4). As shown in context with the 5- and 11-month-old data (Fig. 4A), scavenger receptor transcript level was already significantly elevated above its nontransgenic control as early as at 5 weeks. Figure 4B expresses the data from Figure 4A normalized to the 5-week-old nontransgenic as a control, thereby emphasizing the degree of increase in scavenger receptor mRNA over the course of the progressive glomerulosclerosis. Figure 4B also shows an increased expression level of this receptor in control

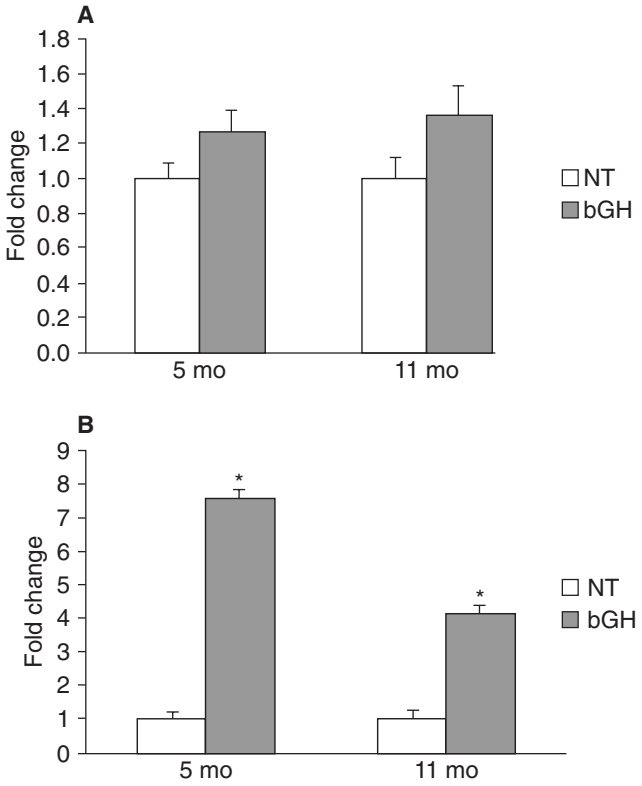

Fig. 3. Relative mRNA expression of low-density lipoprotein (LDL) receptor (*A*) and scavenger receptor (*B*) measured by real-time reverse transcription-polymerase chain reaction (RT-PCR) in kidney cortex of bovine growth hormone (bGH) and nontransgenic (NT) mice at 5 and 11 months of age. There were no significant differences in LDL receptor transcript levels between bGH and age-matched controls at either 5 or 11 months (A). In contrast, scavenger receptor mRNA expression was markedly elevated in bGH compared to nontransgenic in both age groups (B). Bars represent fold change of bGH values over age-matched nontransgenic and are mean ± SEM of three independent experiments. $^*P < 0.05$ vs. age-matched controls.

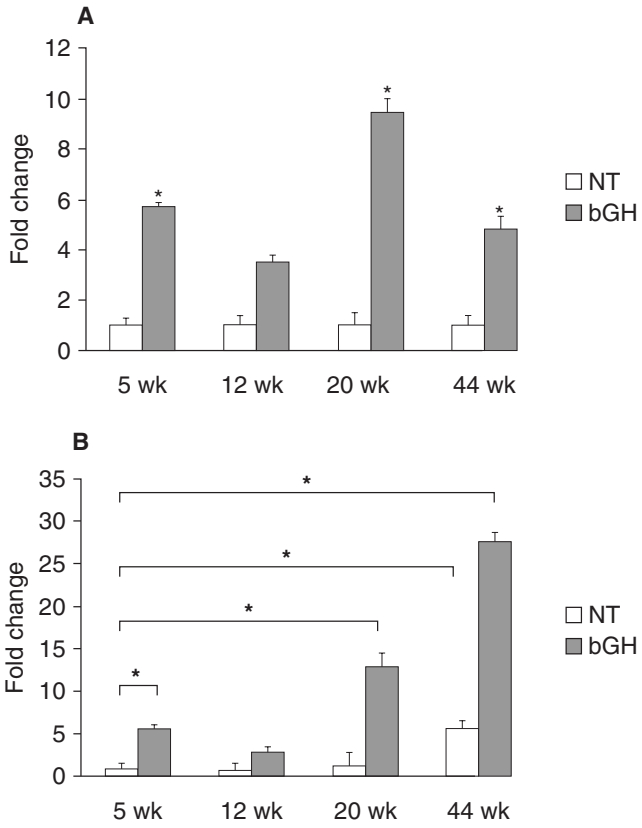

Fig. 4. To study the expression of scavenger receptor throughout the course of glomerulosclerosis, results from earlier time points (5 and 12 weeks) were combined with those of 5 and 11 months (data from Figure 3B, identified in this figure as 20 and 44 weeks). (*A*) Bars represent fold change compared to the respective age-matched control. (*B*) Normalization of scavenger receptor mRNA expression to the average value of control mice at the earliest time point (nontransgenic 5 weeks). Significant differences between groups were determined by analysis of variance (ANOVA) followed by Bonferroni multiple comparisons of ΔC_T values. $^*P < 0.05$ vs. age-matched nontransgenic (A) and vs. 5-week-old nontransgenic (B).

animals at 11 months (44 weeks) compared to the 5-week-old nontransgenic. Protein expression of scavenger receptor was not assessed in this study because the antibodies against this receptor commercially available recognize an epitope that is polymorphic in C57BL/6 mouse strain, from which the bGH and nontransgenic mice strains are derived [32].

DISCUSSION

The bGH transgenic mouse typically exhibits an increased body and kidney weight accompanied by development of progressive glomerulosclerosis, [29, 33, 34]. In the present study, these characteristics were found at both 5 and 11 months of age. In addition, a higher relative kidney weight with severe glomerular lesions (highest glomerulosclerosis scores) at 11 months compared to 5-month-old bGH mice was consistent with the progressive nature of the disease [29, 35].

Despite the elevated serum insulin and the remarkable similarity between the histopathologic characteristics of the growth hormone–induced glomerular lesion and that of diabetic nephropathy, serum glucose remains within a normal range in the bGH mice [8, 29, 35] eliminating a possible contribution of diabetes mellitus in this model of glomerulosclerosis. Serum lipid levels, however, are altered in these animals and have been associated with the elevated concentration of circulating growth hormone in the bGH transgenic mouse [8, 34]. Our present findings furthermore show that serum lipid levels vary with age in the bGH mouse. At 5 months, serum cholesterol was increased compared to age-matched nontransgenic controls. Similar results were shown by other investigators in the bGH mouse at 8 months of age [8]. However, our studies showed that by 11 months, when these mice exhibit a severe degree of renal disease, serum cholesterol had fallen to control levels. We suggest that the temporal variations in serum cholesterol observed here may reflect the combined effects of growth hormone excess as well as hemodynamic and metabolic

changes accompanying the progression of chronic kidney disease.

The role of growth hormone–induced renal disease on serum lipid is complicated, however, since growth hormone has a dual effect on lipid metabolism. Administration of growth hormone to individuals with growth hormone deficiency is beneficial in correcting the severe lipid abnormalities observed in this condition [36–38]. Conversely, supraphysiologic levels of growth hormone (e.g., those occurring in acromegaly) have an opposite effect leading to hyperlipidemia and increased risk for cardiovascular disease [39]. Although both excess growth hormone and chronic kidney disease favor an increase in serum triglycerides [39, 40], a decrease in triglycerides accompanied by a low serum concentration of free fatty acids has been previously reported in the bGH mouse and is corroborated in the present study [8]. The decrease in triglycerides has been attributed to a reduction in synthesis as this abnormality was reversed by administration of a high-fat diet [8].

An increase in serum cholesterol is often seen in the course of clinical and experimental chronic kidney diseases [41–44] demonstrating that renal disease, especially when accompanied by proteinuria plays a role in the pathogenesis of hypercholesterolemia. Conversely, a prospective large-scale clinical investigation involving more than 22,000 apparently healthy males has shown that high circulating cholesterol levels represent a risk for renal disease, supporting the hypothesis that kidney damage may be produced by hypercholesterolemia [45]. Indeed, in both experimental and clinical chronic kidney diseases accompanied by hyperlipidemia, lipid deposits are often in evidence in the mesangium [46, 47]. In the present study, mesangial lipid deposition was increased in bGH mice as early as at 5 weeks of age and was sustained up to 11 months compared to respective controls, and yet circulating cholesterol levels were at normal levels in the 11-month-old animals. The lack of a direct correlation between serum cholesterol level and increased glomerular lipid deposition in our bGH model led us to investigate intrinsic mesangial pathways of lipid synthesis and uptake to explain the increased glomerular lipid accumulation with growth hormone excess.

Increased urinary protein excretion per se has also been associated with direct glomerular damage, in part, through accumulation of lipoperoxidation end products in the glomerulus [48] and thus the role of proteinuria in our present findings is of interest. However, data previously published [29, 35] by other investigators and observations from our laboratory (unpublished) have demonstrated that proteinuria is significantly elevated only at moderate and severe stages of glomerulosclerosis in the bGH model. In the present study, there was no difference in urinary protein content in bGH compared to control mice at 5 weeks of age and yet, glomerular neutral lipid staining was positive at this early point, suggesting that proteinuria does not play a major role in initiating the elevated glomerular lipid accumulation in the bGH model.

Lipid deposits in the kidney have been observed in various types of nephropathies independent of hyperlipidemia and have been associated with increased renal expression of LDL and scavenger receptor, suggesting that local factors play an important role in glomerular lipid accumulation [26]. In the transgenic bGH mouse, bovine growth hormone, in addition to being abnormally high in the circulation, is ectopically expressed at different levels by several organs, including liver and kidney [33, 34], where it may exert significant autocrine effects. Studies performed with 6-month-old bGH mice have shown that expression of several genes, including receptors and enzymes that regulate lipid metabolism, was markedly affected by growth hormone excess in the liver of this mouse model [9].

Our present findings demonstrate that the regulation of lipid metabolism is also altered in the kidney of the bGH mouse and suggest that the high levels of growth hormone lead to glomerular lipid accumulation in this animal model. Regarding the source of the glomerular lipid, cholesterol synthesis, and/or uptake, the present results do not support an influence of growth hormone on LDL receptor in the renal cortex of bGH mouse. This result was surprising in light of our previous observation of growth hormone–induced up-regulation of LDL receptor in mouse mesangial cells in vitro [28]. This may be explained by the fact that the earlier result was obtained with cells cultured in a lipoprotein-deficient medium, which may not reflect the LDL milieu in the glomerulus in the present in vivo study. As for cholesterol synthesis, although an increased HMGR mRNA expression was observed in the bGH renal cortex at 11 months of age, no significant difference was found in HMGR protein expression in the kidney cortex of bGH compared to control mice at either 5 or 11 months of age, suggesting that the contribution of cholesterol synthesis to the glomerular lipid deposition is probably less important than other pathways leading to lipid accumulation. The discrepancy between mRNA and protein expression may have resulted from posttranscriptional regulation of HMGR.

In contrast, scavenger receptor transcript levels, which are not regulated by intracellular lipid feedback mechanisms, were markedly elevated in the bGH mouse (800% above controls at 5 months). The increase above age-matched control fell to fourfold at 11 months largely because of a twofold increase in scavenger receptor expression in the nontransgenic group. Thus, the high level of scavenger receptor expression in the bGH renal cortex was in fact sustained through the course of the disease up to 11 months of age. The study of younger animals

demonstrated that scavenger receptor expression was markedly up-regulated in bGH compared with nontransgenic mice as early as at 5 weeks. The findings of an early occurrence of class A scavenger receptor increase in the bGH model strongly suggest that scavenger receptor up-regulation could be a precipitating event in the observed elevation in glomerular lipid. Apart from the growth hormone influence on scavenger receptor transcript level, the apparent age-related increase in this transcript in control mice raises the possibility that this receptor, in addition to its involvement in experimental models of glomerulosclerosis could play a role in the development of chronic kidney disease in the aging process.

Class A scavenger receptor is macrophage-specific, but is also expressed in other cell types, including smooth muscle [49, 50] and rat mesangial cells [21]. It is noteworthy that human and mouse mesangial cells lose the ability to express this receptor when transferred from in vivo to in vitro conditions [22]. However, it has been shown that in cultured rat mesangial cells, an increased uptake of oxidized LDL by scavenger receptor can lead to the formation of foam cells in vitro [21]. In addition, a body of evidence has shown that scavenger receptor–mediated oxizided LDL uptake plays a major role in foam cell formation and lipid accumulation in chronic kidney diseases [10, 11]. The positive oil red-O staining that we show in the mesangium of bGH mice therefore could reflect either macrophage or mesangial foam cell localization, as seen in diet-induced hyperlipidemic models of glomerulosclerosis [47, 51].

Takemura et al [26], using immunohistochemical techniques, have demonstrated that scavenger receptor was up-regulated in both glomerular epithelial and mesangial cells in various kidney diseases [26]. Our present results suggest that this up-regulation of scavenger receptor in both clinical and experimental glomerular disease may involve an increased presence of scavenger receptor transcripts in the mesangium. However, it remains to be defined whether the elevated transcript represents transcriptional up-regulation of resident cells or whether it represents an increased influx of cells (monocytes/macrophages) expressing a high level of the receptor. There is to our knowledge no data on the growth hormone regulation of class A scavenger receptor expression. Nonetheless, it is of interest that renal cortex scavenger receptor A in the present study responds oppositely to growth hormone than does hepatic class B scavenger receptor in a bGH transgenic mouse model [9].

CONCLUSION

Our present findings of increased scavenger receptor expression in the kidney of bGH mice compared with nontransgenic controls suggest an endocrine and/or autocrine growth hormone effect in the kidney that may help to explain the mesangial lipid deposition in absence of hyperlipidemia in the advanced stages of glomerulosclerosis. Our results further support the hypothesis that growth hormone–induced lipid alterations in the kidney contribute to the development of glomerulosclerosis in the bGH mouse model.

ACKNOWLEDGMENTS

The authors thank Dr. Maria Cecilia Mendonça, Dr. A. Geetha C. Chilakamarri, and Dr. Ajay Verma for the invaluable technical support. Marcos O. Machado received support from CAPES Foundation (Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), Brazil.

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